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Influence of sexual hormones on Chagas disease

Influencia de las hormonas sexuales en la enfermedad de Chagas

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Abstract

Objective: *Analyze sex hormone's influence during Chagas disease.* **Methods:** *Male and female BALB/c mice were divided into six groups, four experimental (sham, orchiectomized, orchiectomized and supplemented with estradiol, orchiectomized supplemented with testosterone, oophorectomized, oophorectomized and supplemented with estradiol, and oophorectomized and supplemented with testosterone), and two control (healthy and intraperitoneally with T. cruzi strain NINOA infected). Clinical data were recorded daily, parasitemia was evaluated using a Neubauer chamber during the infection, and heart histopathological analysis was performed using the paraffin embedding technique. To analyze parasitemia curves and the area under the parametric curves, two-way ANOVA test was performed to correlate groups' data. P-values < 0.05 were considered statistically significant.* **Results:** *Higher mortality rates, cardiomegaly, hepatomegaly, ascites, edema, higher parasitemia levels, more amastigote nests, and more severe inflammatory infiltrate were found in higher testosterone concentration mice, whereas in higher estradiol concentration groups, paresia, prostration, edema, and necrosis were found.* **Conclusions:** *Our results showed that testosterone increased infection severity, whereas estradiol had the opposite effect. This research improves the understanding of sex hormones´ infuence upon this infection to contribute with the handling of Chagas´ disease.*

Keywords: *Chagas disease. Estradiol. Gonadal steroid hormones. Testosterone. Trypanosoma cruzi.*

Resumen

Objetivo: *Analizar la influencia de las hormonas durante la enfermedad de Chagas.* **Métodos:** *Se separaron grupos de ratones macho y hembras BALB/c, todos infectados con T. cruzi (cepa NINOA), 4 grupos experimentales de machos (Sham, orquidectamizados, orquidectimezados y suplementados con estradiol, orquidectamizaos y suplementados con testosterona). 4 grupos experimentales de hembras (oforectomizadas, oforectomizadas y suplementadas con estradiol, oforectomizadas y suplementadas con testosterona y sham), and y dos grupos control para cada sexo (sin infección e infectados intraperitonealmente con T. cruzi (cepa NINOA). Los datos clínicos fueron registrados diariamente, la parasitemia fue evaluada durante toda la infección utilizando una cámara de Neubauer y el análisis histopatológico del corazón fue realizada con la técnica de inclusión en parafina. Para el análisis de las curvas de parasitemia y el área bajo la curva, se realizó una prueba de ANOVA de dos vías, p < 0.05 fueron considerados estadísticamente diferentes.* **Resultados:** *Las mayores tasas de mortalidad, cardiomegalia, hepatomegalia y mayor infiltrado inflamatorio, se encontró en los ratones con una mayor concentración de testosterona. En contraste los ratones con mayor concentración de estradiol presentaron*

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paresia, postración edema y necrosis. **Conclusiones:** *Nuestros resultados ponen en manifiesto que la testosterona incrementa la severidad del curso de la enfermedad de Chagas, mientras que el estradiol tuvo el efecto opuesto. Este trabajo mejora el entendimiento del rol que juegan las hormonas sexuales en esta infección para contribuir en un mejor manejo de la enfermedad de Chagas.*

Palabras clave: *Enfermedad de Chagas. Estradiol. Hormonas esteroideas gonadales. Testosterona. Trypanosoma cruzi.*

Introduction

Chagas disease (CD) is a parasitic zoonosis caused by the protozoan *Trypanosoma cruzi*. Even this disease is endemic of America, nowadays, is considered an emerging worldwide disease affecting nearly 8 million people. CD incidence is 30,000 cases yearly, and currently, is the most lethal parasitosis with 14,000 deaths annually[1].

As Chagas himself said "the virulence of *Schizotrypanum cruzi* (now *Trypanosoma cruzi*) is influenced by several factors, whose nature and effects we do not know yet"[2]. Currently, the factors that determine host susceptibility to *T. cruzi* infection remained unknown.

The host endocrine system plays an important role in the establishment and development of the parasitic infections, importantly the sexual dimorphism (different host susceptibility between males and females)[3]. It has been described that the testosterone (T) increases host susceptibility and decreases the immune system efficiency[4] and, the estradiol (E2) has the opposite effect[5].

In another parasitosis, sexual dimorphism was reported. In leishmaniasis, male-infected hamsters had bigger lesions than females[6], and men are infected more often than women[7]. Hence, better understanding of sexual dimorphism in parasitic diseases will allow the development of more efficacious treatments[7].

Regarding to CD, it has been proposed that the microenvironment of each sex modulates the host response. However, very little is known about it.

Materials and methods

Parasites and animals

T. cruzi NINOA strain, member of the predominant lineage in Mexico, was used in these experiments[8].

Adult female and male BALB/c mice (6 weeks old) were maintained and handled according to the Animal Care and Use Committee Guide[9]. The protocol for this study was evaluated and approved by the Bioethics Committee.

Mice were divided into six animal groups as follows: uninfected or infected intraperitoneally with 1×10^3 trypomastigotes[10] (infected, sham infected, orchiectomized, orchiectomized and supplemented with estradiol, orchiectomized supplemented with testosterone, oophorectomized, oophorectomized and supplemented with estradiol, and oophorectomized and supplemented with testosterone). The sham group were mice that had a simulated operation, they were opened and closed without any organ removal, just to verify if the operation istelf causes any alteration.

Gonadectomy

Oophorectomies and orchiectomies were performed on mice. In addition, fake operations (sham) were performed to account any change that the operation itself could had in *T. cruzi* infection behavior.

Hormones administration

Estradiol (5 mg/kg) (Whitney, Mexico City, Mexico) and testosterone (20 mg/kg) Ara-Test 2500 (Laboratorios Aranda, Mexico City, Mexico) were administrated subcutaneously weekly throughout the experiment (3 months).

Clinical features, parasitemia, and mortality

Clinical features presented during the parasitemia curves were recorded. Parasitemia was quantified according to the modified Brenner' technique employing a Neubauer chamber Blaubrand (Merck, Darmstadt, Germany). Then, parasitemia curves were constructed[11]. Mice death was recorded daily. All experiments were performed in triplicate.

Histopathological analysis

In the parasitemia peak (the day with the most parasites), mice were sacrificed[12]. Then, hearts were obtained, paraffin embedded, and blocks were sliced on a microtome Thermo Scientific HM325 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) to

obtain 30 sections of 5μm (6 slides per heart), slides were stained with the hematoxylin/eosin (HE) technique. For each section, six microscopic fields were counted through a microscope Nikon-Eclipse 50i (Nikon, Chiyoda, Japan) at a magnification of ×40. Amastigote nest numbers were recorded. Inflammatory infiltrate was ranked as follows: absent (–), mild (< 25% of the field), moderate (25-50% of the field), and severe (> 50% of the field).

Statistical analysis

To analyze parasitemia curves and the area under the curve, two-way ANOVA test was performed. $p < 0.05$ was considered statistically significant. Tests were performed using GraphPad Prism v.7 (GraphPad Software, La Jolla, CA, USA).

Results

Clinical features, parasitemia, and mortality

Since the second week post-inoculation (p.i.), parasitemia was positive. On the third week p.i., infected mice presented: piloerection and postration; paresia was presented only in higher estradiol groups. Statiscally, *T. cruzi*-infected and sham *T. cruzi-infected* groups behaved similarly (both sex groups) ($p < 0.05$). Regarding to the females, the oophorectomized *T. cruzi-infected* group had the highest parasitemia peak (1.75×10^5 parasites/mL) at day 17 p.i. contrasting ooporectomized and estradiol supplemented *T. cruzi*-infected group has the lowest (5×10^4 parasites/mL) at day 24 p.i. on the other hand, concerning to malesthe highest parasitemia peak was presented by *T. cruzi*-infected and *T-cruzi* sham infected groups (4.8×10^5 parasites/mL) at day 33 p.i. whereas the lowest peak was presented by orchiectomized (Fig. 1).

To compare the parasitemia, the area under the curve was calculated. Regarding females, oophorectomized *T. cruzi*-infected group presented the highest parasitemia whereas oophorectomized supplemented with estradiol *T. cruzi*-infected group, the lowest. In males, the highest parasitemia was displayed by the *T. cruzi-infected*, while orchiectomized supplemented with estradiol *T. cruzi-infected* group, the lowest. Comparing both sexes, *T. cruzi-infected* and sham *T. cruzi*-infected groups had a similar parasitemia, showing that the fake operation does not change the infection course by itself. The highest parasitemia corresponded to *T. cruzi*-infected and sham *T. cruzi-infected* males; oophorectomized supplemented with estradiol females had the lowest (Fig. 2).

Mortality percentage was higher in males than in females. Regarding to females, deceases were present mainly in the oophorectomized, stradiol supplemented, *T. cruzi*-infected group; whilst in minor amount in the oophorectomized *T. cruzi-infected* group. In contrast, males died on almost all groups (except on the orchiectomized supplemented with estradiol *T. cruzi*-infected group). The highest mortality was presented by orchiectomized supplemented with testosterone *T. cruzi*-infected group (Fig. 3).

Histopathological analysis

Hearts of *T. cruzi*-infected mice showed cardiomegaly. Males showed higher cardiomegaly rate, ascites, and hepatomegaly; females showed more edema and necrosis.

Oophorectomized *T. cruzi*-infected group had more amastigote nests, whilst, orchiectomized supplemented with testosterone *T. cruzi*-infected group had severest inflammatory infiltrate. Orchiectomized *T. cruzi*-infected group had less amastigote nests, and orchiectomized supplemented with estradiol *T. cruzi*-infected group had less inflammatory infiltrate (Fig. 4).

Discussion

The host-parasite relationship is multifactorial; sex hormones could affect infection intensity[13]. Sexual dimorphism has been scarcely reported for CD[14].

Clinically, infected mice from both sex groups presented piloerection caused by adrenergic receptor activation in stressed individuals and prostration that has been described as acute phase CD sign on humans with *T. cruzi*-infection[14]. Both features have been reported with *T. cruzi* Y strain[5], as well as the paresia that leads them to take a hunch posture[15], presented in the mice groups with higher E2 concentration. This could be explained by host autoantibodies denervation, as occurred in the Chagasic hearts[16].

In males, orchiectomized group presented fewer parasites than non-orchiectomized ones as was reported in *T. cruzi* Y strain[5]. In females, higher E2 groups had less parasitemia as has been reported[17].

Heart failure implies cardiomegaly, accompanied by hepatomegaly, ascites, and edema[18], signs presented in the *T. cruzi*-infected mice. Hepatomegaly is a consequence of this organ´s hypoperfusion, in fact, ascites

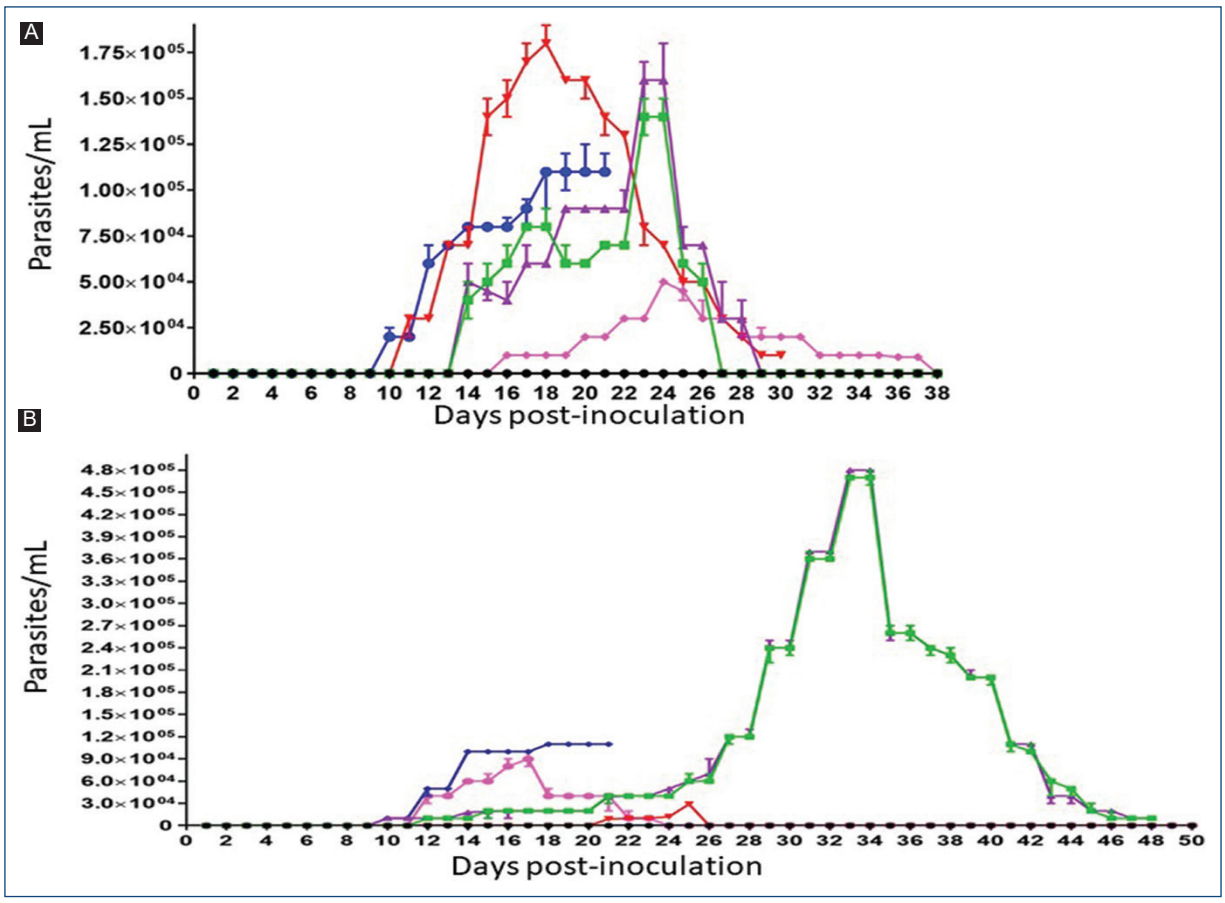

Figure 1. Parasitemia curves of the behavior of *Trypanosoma cruzi* in mice of each mice group among all the experiment; the lines are discontinued if the mice group died. **A:** females. 1. Uninfected (black). 2. *T. cruzi-infected* (green). 3. Sham *T. cruzi-infected* (purple). 4. Oophorectomized *T. cruzi-infected* (red). 5. Oophorectomized and supplemented with estradiol *T. cruzi-infected* (pink). 6. Oophorectomized and supplemented with testosterone *T. cruzi-infected* (blue). **B:** males. 1. Uninfected (black). 2. *T. cruzi-infected* (green). 3. Sham *T. cruzi-infected* (purple). 4. Orchiectomized *T. cruzi-infected* (red). 5. Orchiectomized and supplemented with estradiol *T. cruzi-infected* (pink). 6. Orchiectomized and supplemented with testosterone *T. cruzi*-infected (blue).

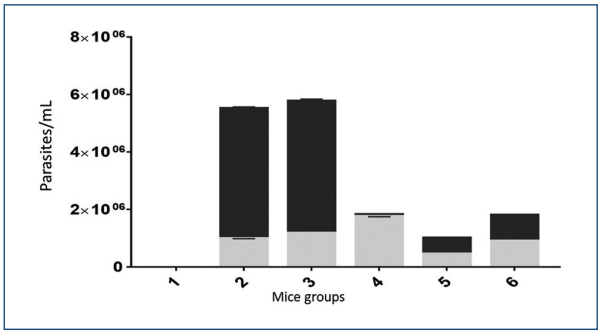

Figure 2. Parasitemia presented in *Trypanosoma cruzi*-infected mice. Males (black) and females (gray). 1. Uninfected, 2. *T. cruzi-infected*. 3. Sham *T. cruzi-infected*. 4. Gonadectomized *T. cruzi-infected*. 5. Gonadectomized and supplemented with estradiol *T. cruzi-infected*. 6. Gonadectomized and supplemented with testosterone *T. cruzi-infected*.

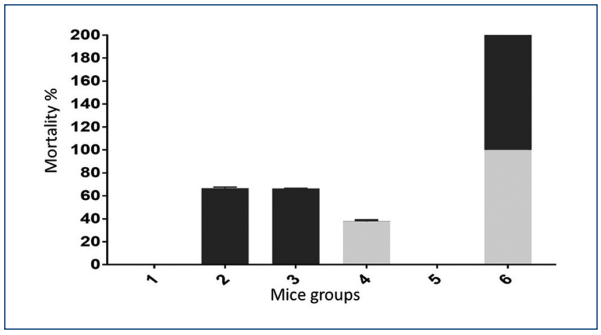

Figure 3. Mortality of the *Trypanosoma cruzi*-infected mice. Males (black) and females (gray). 1. Uninfected. 2. *T. cruzi-infected*. 3. Sham *T. cruzi*-infected. 4. Gonadectomized *T. cruzi*-infected. 5. Gonadectomized and supplemented with estradiol *T. cruzi*-infected. 6. Gonadectomized and supplemented with testosterone *T. cruzi*-infected.

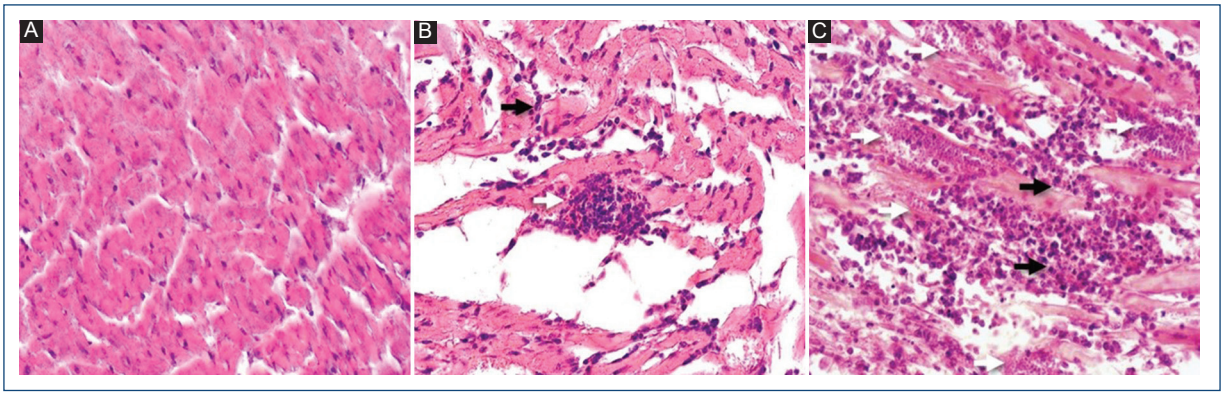

Figure 4. Microscopic analysis of the heart tissues of *Trypanosoma cruzi*-infected mice. Heart tissues were stained according to the HE technique to show the *T. cruzi* amastigote nests NINOA strain, edema, and the inflammatory infiltrate. The photograph was taken at ×40 magnification. **A:** healthy mice tissue. **B:** heart tissue *T. cruzi*-infected female mouse, amastigote nest, and mild acute inflammatory infiltrate. **C:** heart tissue *T. cruzi*-infected male mouse, amastigote nests, edema, and severe acute inflammatory infiltrate. Amastigote nest (white arrow); inflammatory infiltrate (black arrow).

accompanies this sign on 25% of the cases[19]. Inflammation causes edema[20] and necrosis, resulted from ischemia produced by the reactive oxygen species (ROS) released in the inflammatory process.

In terms of mortality, higher T concentration correlated with a higher mortality rate accordingly with Tulahuén strain *T. cruzi*-infected reports[21]. In CD Mexican reports, most of the patients are women[13]. It has been proposed that the parasites have a preference to infect the sex that lives longer[22]; further, we propose that men, due to the higher death rates (curiously related to cardiovascular diseases), die before a proper CD diagnosis could be done.

Higher T concentration groups presented more severe inflammatory infiltrate; accordingly, cardiac damage may be a consequence more than the presence of the parasite itself, of the inflammatory oxidative damage[21]. Besides, *T. cruzi* infection disturbed mitochondrial membrane, leading to ROS production, that oxidize lipids, proteins, and deoxyribonucleic acid (DNA)[23].

In this regard, estrogens are a cardiovascular protector factor because they can decrease the NO (potent vasodilator) concentration[21], helping the host. In contrast, the male immune system could be weaker due to the oxidation handicap hypothesis (OHH), which postulates that males have a higher T concentration, implying an overproduction of ROS, making them more susceptible to tissue damage[24].

Moreover, host´s cells mitochondrial antioxidant function is lower in males[23], and antioxidant enzymes, superoxide dismutase and catalase, concentrations are host sex-related (83% males versus 180% females)[25].

It has been reported that T concentration does not influence the severity of the cardiac damage[26] contrasting with the present study where T does influence it.

Thus, in CD, inflammation plays a main role in tissue damage, and in patients´ death, because this protozoan avoids the oxidant action, but the host cell does not[27]. Sex hormones have been related to this because can control the macrophages[21]; the feminine supremacy paradigm stablishes that the female hosts are more resistant to parasitosis because they have a different immune response[17]; for example, macrophages have estrogen receptors (ERα) that modulate the oxidative microenvironment[28]. Moreover, E2 inhibits M1 macrophage activation (by inhibiting the metalloprotease 9)[29] having an anti-inflammatory activity, but leads to the interleukin 10 production and to the chronicity of the infection[30].

Notwithstanding, as *T. cruzi* affects cytokine release in the host, this parasite could modulate macrophage´s polarization in CD and this process could be sex hormone mediated. Therefore, experiments are being carried out in this regard.

Conclusions

The data presented showed that testosterone increased infection severity, whereas estradiol had the opposite effect, not only confirm previous data but also

expanding and detailing these phenomena, highlighting the importance of *T. cruzi* host sex bias. This research improves the understanding of sex hormones´ infuence upon this infection to contribute with the handling of Chagas disease.

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Conflicts of interest

None.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Use of artificial intelligence for generating text. The authors declare that they have not used any type of generative artificial intelligence for the writing of this manuscript, nor for the creation of images, graphics, tables, or their corresponding captions.

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